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(21) International Application Number: <b>PCT/US97/05101</b> (22) International Filing Date: <b>27 March 1997 (27.03.97)</b> (30) Priority Data: 08/624,914                      27 March 1996 (27.03.96)                      US (71) Applicant (for all designated States except US): <b>INSPIRE PHARMACEUTICALS, INC. [-/US]; Suite 470, 4222 Emperor Boulevard, Durham, NC 27703 (US).</b> (72) Inventors; and (75) Inventors/Applicants (for US only): <b>JACOBUS, Karla, M. [-/US]; 200 Riverwalk Circle, Carv, NC 27511 (US). YERXA, Ben [-/US]; 1330 Hathaway Road, Raleigh, NC 27608 (US). PENDERGAST, William [-/US]; 5815 Williamsburg Way, Durham, NC 27713 (US). BOUCHER, Ricard, C., Jr. [-/US]; 735 Girthout Road, Chapel Hill, NC 27514 (US). RIDEOUT, Janet, L. [-/US]; 3101 Morningside Drive, Raleigh, NC 27607 (US). DRUTZ, David, J. [-/US]; 1059 Carterbury Lane, Chapel Hill, NC 27514 (US). JAMES, Michael, K. [-/US]; 4329 Basal Creek Lane, Fuquay-Varina, NC 27526 (US). STUTTS, Monroe, Jackson [-/US]; 104 Morgan Bluff Lane, Chapel Hill, NC 27514 (US). GEARY, Cary [-/US]; 7011 Thurston-Bowles, Campus Box 7248, UNC at Chapel Hill, Chapel Hill, NC 27599</b>		(US). LAZAROWSKI, Eduardo, R. [-/US]; Apartment #3, 212 Pinegate, Chapel Hill, NC 27514 (US). (74) Agents: <b>DREHKOFF, W., Dennis et al.; Banner &amp; Witcoff, Ltd., 10 South Wacker Drive, Chicago, IL 60606 (US).</b> (81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b> Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: <b>METHOD OF TREATING CILIARY DYSKINESIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS</b> (57) Abstract <p>A method of stimulating ciliary beat frequency in a subject in need of such treatment is disclosed. The method comprises administering to the airways, ears, eyes, or genito-urinary tract of the subject a triphosphate nucleotide such as uridine 5'-triphosphate (UTP), an analog of UTP, or any other analog, in an amount effective to stimulate ciliary beat frequency. This method is useful for treating patients afflicted with ciliary dyskinesia, Kartagener's syndrome, or any other disease involving dysfunction of ciliary movement, such as male infertility caused by impairment of propulsion of the spermatozoa or immune deficiency caused by impairment of ciliary movement in neutrophils or macrophages. Pharmaceutical formulations and methods of making the same are also disclosed. Methods of administering the same would include any liquid suspension (including nasal spray or nasal or eye drops), oral, inhaled by nebulization, topical, injected, suppository, intra-operative by instillation or application, or ex vivo direct application to spermatozoa.</p>		

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**METHOD OF TREATING CILIARY DYSKINESIA  
WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS**

5

**INTRODUCTION**

**Technical Field**

This invention relates to a method of stimulating ciliary beat frequency to promote mucociliary or cough clearance of retained mucus secretions from the lungs, sinuses, or ears of a patient by administering certain uridine, adenosine, or cytidine triphosphates.

**Background of the Invention**

Mucociliary clearance is an important defense mechanism of the human airway and middle/inner ear tract. Coordinated beats of cilia in the nose, trachea, bronchi, and middle ear propel the mucous layer toward the pharynx, carrying along with it microorganisms and other particles captured in the mucus. Normal function of this system depends on the frequency and coordination of ciliary beating and the properties of mucus. There are three components of the mucociliary clearance system: (1) the mucin layer, which is formed by secretion of mucins by goblet cells, (2) cilia, which transport the overlying mucin layer by synchronous beating, and (3) the periciliary liquid layer, which surrounds the cilia and is less viscous than the mucin layer, allowing free movement of the cilia. The electrolyte and water concentration of the periciliary layer is regulated by the luminal epithelial cells. (R. Boucher, et al., Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology, p. 525-32 entitled "Mechanisms and Therapeutic Actions of Uridine Triphosphates in the Lung" (L. Belardinelli, et al. ed., Alunwer Academic Publishers, Boston 1995)).

Primary ciliary dyskinesia (PCD) is a congenital disease characterized by ultrastructural defects and motility disturbances of cilia, resulting in either absent or abnormal ciliary movement. The most common clinical manifestations of PCD are chronic respiratory disease (e.g., sinusitis, rhinitis, and bronchiectasis) and otitis media. Because PCD patients have either absent or severely impaired mucociliary clearance (MCC), the only available mechanism to clear or move secretions is cough. Cough clearance may be measured in a manner similar to that previously

described for MCC. PCD also impairs the propulsion of spermatozoa, resulting in male infertility. (D. Schidlow, *Ann Allergy* 73(b), 457-68 (1995)). PCD also results in the impairment of cell motility of certain immune system cells, including neutrophils and macrophages. (N. Valerius, *Eur J Clin Invest* 13, 489-94 (1983)). PCD may be responsible for a form of hydrocephalus caused by ciliary malfunction. (M. Greenstone, *Arch Dis Child* 59, 481-82 (1984)). The incidence of PCD has been calculated to be one in 16,000 live births, and an estimated 50% of affected individuals also have situs inversus (dextrocardia). The triad of bronchiectasis, sinusitis, and situs inversus (dextrocardia) is referred to as Kartagener's syndrome. (M. Sleight, *Lancet* ii,476 (1981)). It has been hypothesized that Kartagener's syndrome is caused by a lack of embryonic ciliary movement, resulting in the random rotation of the archenteron such that in half the cases there is situs inversus (dextrocardia) and in the other half there is normal cardia situs. (B. Afzelius *Science* 193, 317-19 (1976)). The clinical course of PCD is characterized primarily by sinus and ear infections early in life with a progressive change to lung/lower airways diseases in adulthood. Chronic airways infections can lead to chronic obstructive changes in the pulmonary tissue, progressive loss of pulmonary function, and eventually death.

A secondary and more common form of ciliary dyskinesia is the acquired form of the disease. Chronic inflammation caused by severe viral or bacterial respiratory infections, chronic smoking, severe air pollution, chemical or thermal burns to the airways, intubation and mechanical ventilation, and near drowning can result in changes in ciliary structure including disruption of the cellular membrane, loss or incorporation of microtubules, and formation of compound cilia, all of which can result in abnormal or absent ciliary function. (J. Ballenger *Ann Otol Rhinol Laryngol* 97 (3 Pt. 1), 253-58 (1988); M. Pedersen *Lung* 168 Suppl., 368-76 (1990)). Respiratory infections which often lead to secondary ciliary dyskinesia include influenza, adult respiratory distress syndrome, and ventilator-associated pneumonia (VAP) in intensive care unit (ICU) patients. In some cases acquired ciliary dyskinesia may be reversed with appropriate and timely intervention; however, permanent damage and/or sustained exposure to the above factors may render the ciliary damage irreversible. The clinical manifestations and course would likely appear similar to PCD with respect to chronic lung infections, progressive loss of pulmonary function, and obstructive pulmonary disease.

The typical mammalian respiratory epithelial cell contains about 200 cilia. Each cilium has nine peripheral microtubular doublets and two central tubules. Each peripheral doublet contains an A subunit and a B subunit, and each A subunit has a set of curved arms attached to it called the inner and outer dynein arms. These dynein arms contain ATPase--an enzyme which breaks down adenosine triphosphate (ATP), providing the energy for ciliary movement. Because the most common ultrastructural abnormality associated with primary ciliary dyskinesia is the total absence of dynein arms (B. Afzelius, et al., *J Cell Biol* 66, 225-32 (1975)), researchers began investigating whether extracellular application of ATP and ATPase could activate immotile cilia in vitro. (J. Forrest, et al., *Am Rev Resp Dis* 120, 511-15 (1979)). Although the results appeared positive, the findings have not been consistently reproduced by others. It was later discovered that extracellular application of  $Ca^{2+}$  and cAMP could increase the beat frequency of respiratory tract cilia. (A. Lansley, et al., *Am J. Physiol* 263, L232-42 (1992)). It has not been definitively established that any therapy will stimulate cilia beat in cases where complete ciliary immotility has been demonstrated. In such cases, it might be of therapeutic benefit to increase hydration of the viscous mucous secretions.

Applicant has discovered that extracellular triphosphate nucleotides, especially uridine 5'-triphosphate (UTP) modulates all three components of the mucociliary transport system. UTP stimulates ciliary beat frequency (R. Boucher, et al., *supra*); UTP stimulates mucin secretion by goblet cells (M. Lethem, et al., *Am J Respir Cell Mol Biol* 9, 315-22 (1993)); and UTP stimulates  $Cl^{-}$  secretion in airway epithelial cells, which increases hydration of the periciliary liquid layer (M. Knowles, et al., *N Eng J. Med* 325, 533-38 (1991)). Applicant has also demonstrated that UTP is safe and improves cough clearance in PCD patients. (P. Noone, et al., abstract submitted to the 1996 International Conference of The American Thoracic Society).

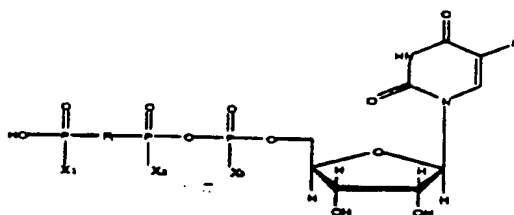
In summary, a variety of clinical manifestations of ciliary dyskinesia, such as absent or impaired mucociliary clearance in the respiratory and middle/inner ear tract, impaired propulsion of spermatozoa, and impaired motility of neutrophils and macrophages can be improved or alleviated by administering UTP and its related compounds, as well as other nucleoside phosphates such as: adenosine 5'-triphosphate (ATP); cytidine 5'-triphosphate (CTP); 1,N<sup>6</sup>-ethenoadenosine triphosphate; adenosine 1-oxide triphosphate; 3,N<sup>4</sup>-ethenocytidine

triphosphate;  $P_1, P_4$ -di(adenosine-5') tetraphosphate ( $A_2P_4$ ); or  $P_1, P_4$ -di(uridine-5') tetraphosphate ( $U_2P_4$ ) to the affected part of the body.

# SUMMARY OF THE INVENTION

A method of treating ciliary dyskinesia in a subject in need of such treatment is disclosed. The method comprises administering to the patient a compound of Formula I, or a pharmaceutically acceptable salt thereof, in an amount effective to stimulate ciliary beat frequency, where possible, in the luminal epithelial cells of the lung or middle/inner ear, eyes, genito-urinary tract; spermatozoa cells; or certain cells of the immune system, including neutrophils and macrophages:

## Formula I



wherein:

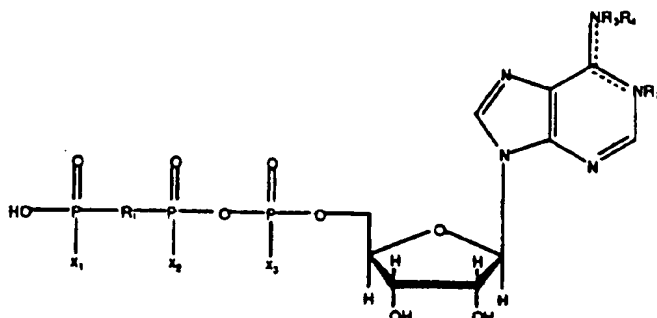
$X_1$ ,  $X_2$ , and  $X_3$  are each independently either  $O^-$  or  $S^-$ .

Preferably,  $X_2$  and  $X_3$  are  $O^-$ .

$R_1$  is O, imido, methylene, or dihalomethylene (e.g., dichloromethylene, difluoromethylene). Preferably,  $R_1$  is oxygen or difluoromethylene.

$R_2$  is H or Br. Preferably,  $R_2$  is H. Particularly preferred compounds of Formula I are uridine 5'-triphosphate (UTP) and uridine 5'-O-(3-thiotriphosphate) (UTP $\gamma$ S).

Formula I is the preferred embodiment of the compound, however, the method of the present invention can also include administering a compound of Formula II (adenosine 5' triphosphate [ATP] or 1,N<sup>6</sup>-ethenoadenosine triphosphate or adenosine 1-oxide triphosphate), or Formula III (cytidine 5' triphosphate [CTP] or 3,N<sup>4</sup>-ethenocytidine triphosphate), or Formula IV (P<sup>1</sup>,P<sup>4</sup>-di(adenosine-5') tetraphosphate (A<sub>2</sub>P<sub>4</sub>) or P<sup>1</sup>,P<sup>4</sup> di(uridine-5') tetraphosphate (U<sub>2</sub>P<sub>4</sub>).

Formula II

5

wherein:

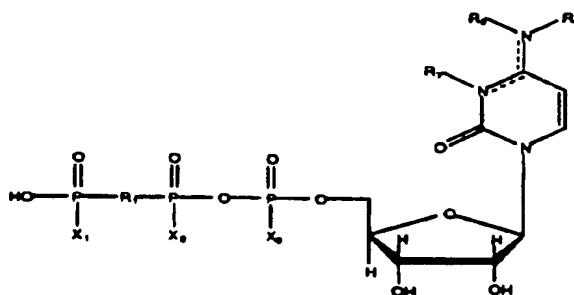
$R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I.

$R_3$  and  $R_4$  are H while  $R_2$  is nothing and there is a double bond between N-1 and C-6 (adenine), or

$R_3$  and  $R_4$  are H while  $R_2$  is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

$R_3$ ,  $R_4$  and  $R_2$  taken together are  $-CH=CH-$ , forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1,N<sup>6</sup>-ethenoadenine).

15

Formula III

20

wherein:

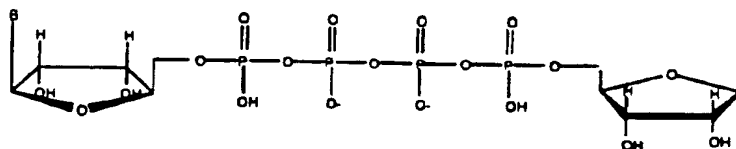
$R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I.

$R_5$  and  $R_6$  are H while  $R_7$  is nothing and there is a double bond between N-3 and C-4 (cytosine), or,



$R_5$ ,  $R_6$  and  $R_7$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3,N<sup>4</sup>-ethenocytosine).

5

Formula IV

wherein:

10

B is adenine or uracil.

15

A second aspect of the present invention is a pharmaceutical formulation containing the compound of Formula I, II, III, or IV in an amount effective to stimulate ciliary beat frequency in: the epithelial cells of the lungs or middle and inner ears; the mucous clearance defense system of the eyes or genito-urinary tract; spermatozoa cells; the ovaries or fallopian tubes; or certain cells of the immune system, including neutrophils and macrophages, in a pharmaceutically acceptable carrier.

20

A third aspect of the present invention is the use of the active compounds disclosed herein for the manufacture of a medicament for the therapeutic stimulation of ciliary beat frequency in: the epithelial cells of the lungs or middle and inner ears; the mucous clearance defense system of the eyes or genito-urinary tract; spermatozoa cells; the ovaries or fallopian tubes; or certain cells of the immune system, including neutrophils and macrophages, of a patient in need of such treatment.

25

### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The method of the present invention may be used to stimulate ciliary beat frequency in a subject in need of such treatment for any reason, including (but not limited to) increasing the mucociliary clearance of retained secretions in the lungs, sinuses, or middle and inner ears. The method of the present invention may also be used to treat primary ciliary dyskinesia, secondary ciliary dyskinesia, Kartagener's syndrome, otitis media, cystic fibrosis, diseases involving the dysfunction of the ocular or genito-urinary mucociliary clearance defense system caused by impairment of ciliary movement, diseases of the immune system caused by impairment of ciliary movement of neutrophils and macrophages, hydrocephalus caused by impairment of ciliary movement, male infertility caused by impairment of the ciliary propulsion of the spermatozoa, female infertility caused by impairment of ciliary movement on the luminal epithelial cells of the ovaries or fallopian tubes, or any other disease caused by an impairment of ciliary movement. The present invention increases mucociliary clearance in three ways: (1) by increasing the ciliary beat frequency of cilia on the surface of luminal epithelial cells, (2) by increasing the secretions of mucins by goblet cells, and (3) by increasing the secretion of water into the periciliary liquid layer by luminal epithelial cells. The mucins secreted by goblet cells form a layer on top of the cilia and captures foreign particles, including viruses and bacteria; the mucin layer is transported by the wave-like action of cilia, and the movement of cilia is facilitated by the composition and hydration of the periciliary liquid layer surrounding the cilia. Although the primary aspect of the present invention is to increase ciliary beat frequency in patients afflicted with ciliary dyskinesia, in patients whose cilia are permanently incapable of any movement regardless of treatment, the active compounds of the present invention can still facilitate the clearance of retained mucous secretions by increasing the secretion of water into the periciliary liquid layer and by increasing the secretion of mucins by goblet cells.

Additionally, because of the well-demonstrated ability of the active compounds of the present invention to enhance lung mucociliary clearance in normal subjects, the active compounds of the present invention can accelerate the clearance of any type of inhaled foreign materials from the airways. This would prove beneficial in a number of

situations--biological warfare, e.g. the chemical warfare agent ricin; smoke inhalation; industrial exposure to inhaled toxins (resulting in e.g., silicosis, anthracosis, and the gamut of so-called pneumoconioses); and allergic reaction to inhaled particles such as pollen.

5 Furthermore, the ability of the active compounds of the present invention to increase lung clearance would also prove beneficial in the diagnosis of lung disease--specifically, to improve the quality of radioisotopic scans of the lungs by removing the secretions that might otherwise obscure the visualization of ventilated portions of the lung. In  
10 radioisotopic lung scanning, the mismatch of ventilated versus perfused lung is used to identify areas of pulmonary infarction. As a result of improved aeration of the lungs after administering the active compounds of the present invention, the ventilated portions of the scan would be more distinct, and the diagnostician would be in a better position to clearly  
15 identify true mismatches.

The present invention is concerned primarily with the treatment of human subjects, but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

20 Compounds illustrative of the compounds of Formula I above include: (a) uridine 5'-triphosphate (UTP); (b) uridine 5'-O-(3-thiotriphosphate) (UTPyS); and (c) 5-bromo-uridine 5'-triphosphate (5-BrUTP). These compounds are known or may be made in accordance with known procedures, or variations thereof which will be apparent to those  
25 skilled in the art. See generally N. Cusack and S. Hourani, *Annals N.Y. Acad. Sci.* 603, 172-81 (entitled "Biological Actions of Extracellular ATP"). For example, UTP may be made in the manner described in Kenner, et al., *J. Chem. Soc.* 1954, 2288; or Hall and Khorana, *J. Am. Chem. Soc.* 76, 5056 (1954). See Merck Index, Monograph No. 9795 (11th Ed. 1989). UTPyS may  
30 be made in the manner described in R. S. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

For simplicity, Formulae I-IV herein illustrate the active compounds in the naturally occurring D-configuration, but the present invention also encompasses compounds in the L-configuration, and  
35 mixtures of compounds in the D- and L- configurations, unless otherwise specified. The naturally occurring D-configuration is preferred.

Compounds illustrative of the compounds of Formula II above include (a) adenosine 5'-triphosphate (ATP) and (b) I,N<sup>6</sup>-

ethenoadenosine triphosphate. Compounds illustrative of the compounds of Formula III above include (a) cytidine 5'-triphosphate and (b) 3,N<sup>4</sup>-ethenocytidine triphosphate. These compounds can be made in accordance with known procedures, or variations thereof which will be apparent to those skilled in the art. For example, phosphorylation of nucleosides by standard methods such as D. Hoard and D. Ott, *J. Am. Chem. Soc.* 87, 1785-1788 (1965); M. Yoshikawa, et al., *Tetrahedron Lett.* 5065-68 (1967) and *idem.*, *Bull. Chem. Soc. (Jpn)* 42, 3505-08 (1969); J. Moffatt and H. Khorana, *J. Am. Chem. Soc.* 83, 649-59 (1961); and B. Fischer, et al., *J. Med. Chem.* 36, 3937-46 (1993) and references therein. Etheno derivatives of cytidine and adenosine are prepared by known methods such as: N. Kotchetkov, et al., *Tetrahedron Lett.* 1993 (1971); J. Barrio, et al., *Biochem. Biophys. Res. Commun.* 46, 597 (1972); J. Secrist, et al., *Biochemistry* 11, 3499 (1972); J. Bierndt, et al., *Nucleic Acids Res.* 5, 789 (1978); K. Koyasuga-Mikado, et al., *Chem. Pharm. Bull. (Tokyo)* 28, 932 (1980). Derivatives with alpha, beta and gamma thiophosphorus groups can be derived by the following or by adapting methods of: J. Ludwig and F. Eckstein, *J. Org. Chem.* 54, 631-35 (1989); F. Eckstein and R. Goody, *Biochemistry* 15, 1685 (1976); R. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

Compounds of Formulas I, II, or III where R<sub>1</sub> is CCl<sub>2</sub> and CF<sub>2</sub> can be prepared by methods similar to that described in G. Blackburn, et al., *J. Chem. Soc. Perkin Trans. I*, 1119-25 (1984). Compounds of Formula I, II, III where R<sub>1</sub> is CH<sub>2</sub> can be prepared by methods similar to that described in T. Myers, et al., *J. Am. Chem. Soc.* 85, 3292-95 (1963).

Compounds illustrative of the compounds of Formula IV include (P<sup>1</sup>,P<sup>4</sup>-di(adenosine-5')) tetraphosphate (A<sub>2</sub>P<sub>4</sub>) or P<sup>1</sup>,P<sup>4</sup>-di(uridine-5') tetraphosphate (U<sub>2</sub>P<sub>4</sub>). These compounds can be made in accordance with known procedures, or variations thereof which will be described by: P. Zamecnik, et al., *Proc. Natl. Acad. Sci. USA* 89, 838-42 (1981); and K. Ng and L. E. Orgel, *Nucleic Acids Res.* 15 (8), 3572-80 (1987).

In addition, UTP, ATP, CTP, A<sub>2</sub>P<sub>4</sub>, 3,N<sup>4</sup>-ethenocytidine triphosphate, 1,N<sup>6</sup>-ethenoadenine triphosphate, adenosine 1-oxide triphosphate, ATP<sub>γ</sub>S, ATP<sub>β</sub>S, ATP<sub>α</sub>S, AMPPCH<sub>2</sub>P, AMPPNHP, N<sup>4</sup>-ethenocytidine and 1,N<sup>6</sup>-ethenoadenosine are commercially available, for example, from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178.

The active compounds of Formulae I - IV may be administered by themselves or in the form of their pharmaceutically acceptable salts, e.g., an alkali metal salt such as sodium or potassium, an alkaline earth metal salts such as manganese, magnesium and calcium or  
5 an ammonium and tetraalkyl ammonium salts,  $NX_4^+$  (wherein X is  $C_{1-4}$  alkyl group). Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

The active compounds disclosed herein may be administered  
10 to the lungs, sinuses, ears, eyes, spermatozoa, ovaries or fallopian tubes, or genito-urinary tract by a variety of suitable means, but are preferably administered by administering a nebulized form of the active compound into their respiratory tract, such that the active compound enters the lungs and reaches the area of the body afflicted with impaired ciliary movement  
15 either directly or via systemic absorption and circulation. The active compound can be aerosolized in a variety of forms, such as, but not limited to, dry powder inhalants, metered dose inhalants, or liquid/liquid suspensions. In dry powder delivery, the UTP may be formulated alone or in combination with diluent or carrier, such as sugars (e.g., lactose,  
20 sucrose, trehalose, mannitol) where the compounds may be intimately incorporated in the matrix through glassification or simply admixed with the carrier, or other acceptable excipients for lung or airway delivery. The dry powder may be obtained by methods known in the art, such as spray-drying, milling, freeze-drying, super-critical fluid manufacturing or via  
25 controlled crystallization or precipitation

The dosage of active compound to stimulate ciliary beat frequency will vary depending on the condition being treated and the state of the subject, but generally an effective amount is the amount sufficient to achieve concentrations of active compound on the lungs, sinuses, ears,  
30 eyes, or genito-urinary surfaces of the subject of from about  $10^{-7}$  to about  $10^{-1}$  Moles/liter, and more preferably from about  $10^{-6}$  to about  $10^{-1}$  Moles/liter.

Depending upon the solubility of the particular formulation of active compound administered, the daily dose to promote fluid  
35 drainage may be divided among one or several unit dose administrations. Preferably, the daily dose is no more than four times per day.

Another means of administering the active compound to the lungs, sinuses, ears, eyes, or genito-urinary tract of the patient to promote

fluid/secretion drainage may include any oral form of the active compound, administered to the patient either by means of a liquid suspension of the active compound which is poured into the mouth of the patient, or by means of a pill form swallowed by the patient.

5 Another means of administering an effective amount of the active compound to the lungs, sinuses, eyes, or middle and inner ears would involve administering a liquid/liquid suspension (either a nasal spray of respirable particles which the subject inhales, or nasal drops of a liquid formulation, or eye drops of a liquid formulation) comprised of the  
10 active compound. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal or eye drops may be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

15 Another means of administering the active compound to the middle ear or eye would include any topical form of the active compound, administered as a cream or gel to the outer ear or eye, which would subsequently permeate through the tympanic membrane or cornea into the middle ear or lens of the patient.

20 Another means of administering the active compound to the middle ear, genito-urinary tract, or eye would involve an injected form of the active compound, injected from the outer ear directly through the tympanic membrane into the middle ear, injected into the genito-urinary tract, or injected into the eye. This could involve a patch containing UTP  
25 which would be applied directly to the tympanic membrane.

Another means of administering the active compound to the lungs, sinuses, middle and inner ears, eyes, or genito-urinary tract would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the lungs,  
30 sinuses, middle ear, eye, genito-urinary tract, or male or female reproductive systems via systemic absorption.

Another means of administering the active compound would involve intra-operative instillation of a gel, cream, or liquid suspension form of the active compound such that a therapeutically  
35 effective amount reaches the lungs, sinuses, middle and inner ears, eyes, or genito-urinary tract.

An additional means of administering the active compound would involve ex vivo administration of the active compound to the

spermatozoa by means of a topical, injection, or immersion form of the compound, such that a therapeutically effective amount of said compound contacts the spermatozoa having impaired ciliary movement.

- 5           An additional means of administering the active compound would involve administration of the active compound via a transdermal patch, in which the active compound would be delivered to the affected are via local absorption or systemic absorption and circulation.

- 10           UTP and compounds for Formulae I - IV also have therapeutic benefit when used in combination with other agents used to treat ciliary dyskinesia, such as, but not limited to: antibiotics; vaccines; decongestants, mucolytic agents; nonsteroidal antiinflammatory agents; steroids; antiviral agents; and bronchodilators. UTP may also be used in combination with other treatments under development, such as gene  
15           therapy. UTP may also be used in combination with the recently discovered therapeutic protein defensin.

The present invention is explained in greater detail in the Examples which follow. These examples are intended as illustrative of the invention, and are not to be taken as limiting thereof.

## EXPERIMENTAL

### Example 1

#### In Vitro Stimulation of Ciliary Beat Frequency

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$10^{-4}$  M UTP was applied to isolated airway epithelial cells from normal subjects. Within four minutes, ciliary beat frequency (CBF) increased by  $76 \pm 17\%$  as compared to baseline (from  $9.3 \pm 0.23$  to  $16.12 \pm 0.92$  Hz,  $n = 7$ ,  $p < 0.0001$ ). Similar results were obtained when  $10^{-4}$  M UTP was applied to isolated airway epithelial cells from patients afflicted with cystic fibrosis. CBF increased by  $56 \pm 17\%$  as compared to baseline (from  $11.25 \pm 0.56$  Hz to  $16.1 \pm 1.45$  Hz).

### Example 2

#### Treatment of Primary Ciliary Dyskinesia

Uridine 5'-triphosphate (UTP) was administered to patients diagnosed with primary ciliary dyskinesia (PCD) (verified by electron microscopy analysis of ciliary ultrastructure defect from nasal biopsy). The efficacy of UTP was determined by measuring the clearance of an inhaled radiolabeled particle from the lung by radionuclide scanning techniques using a gamma camera. Each subject inhaled an aerosol of iron oxide labeled with Technetium 99m ( $^{99}\text{Tc-Fe}_2\text{O}_3$ ). Subjects inhaled the aerosol for approximately 5 minutes. Subjects were then seated in front of a gamma camera, and for the next 20 minutes subjects randomly inhaled either a saline control (0.12% saline), or  $10^{-2}$  M UTP for approximately 20 minutes. After this inhalation, subjects remained seated in front of the gamma camera for the next 30 minutes to measure clearance of the radiolabeled iron oxide. The efficacy of aerosolized UTP in treating primary ciliary dyskinesia was demonstrated by an improvement in cough clearance of Technetium 99m as compared to the saline vehicle alone.

In the same study, the amount of sputum induced by the inhalation of UTP versus placebo was measured. Subjects inhaled either placebo or UTP for 20 minutes according to the method above. For the next 30 minutes, subjects performed 60-90 controlled coughs, and matched sputum samples were collected from 8 of the 12 patients. Total volume of sputum was measured.



Safety data was collected by monitoring heart rate, ECG rhythm strip-Lead II, blood pressure, oxyhemoglobin saturation by pulse oximetry prior to, during, and after inhalation for all dosing periods. All patients during all phases of the study were monitored for any adverse reactions during each dosing period, beginning with inhalation of study drug and ending after the 30-minute scan at 24 hours.

#### Results:

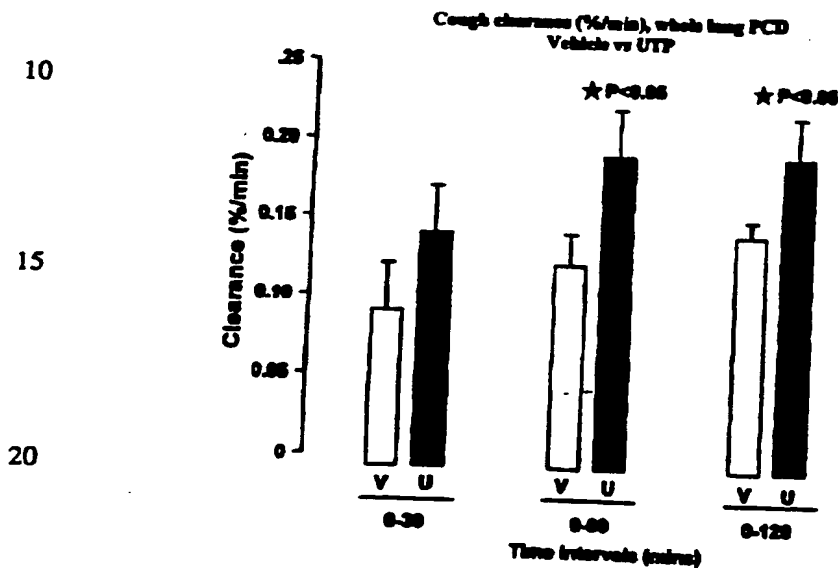


Table I.  
Cough Clearance (%/min), whole lung PCD  
Vehicle vs UTP

This was a randomized, double-blind, placebo-controlled, crossover study in 12 patients with PCD, ages 10 and above. Table I above summarizes data demonstrating that UTP significantly enhanced cough clearance at the 60-minute ( $p < 0.05$ ) and 120-minute ( $p < 0.05$ ) time points, as compared to placebo (vehicle=V); this improvement also approached significance at the 30-minute time point. These data are particularly compelling, given that the effect was observed following only a single dose of UTP. With the data in Table II, these data suggest that there is a strong correlation between measures of cough clearance and sputum expectoration.

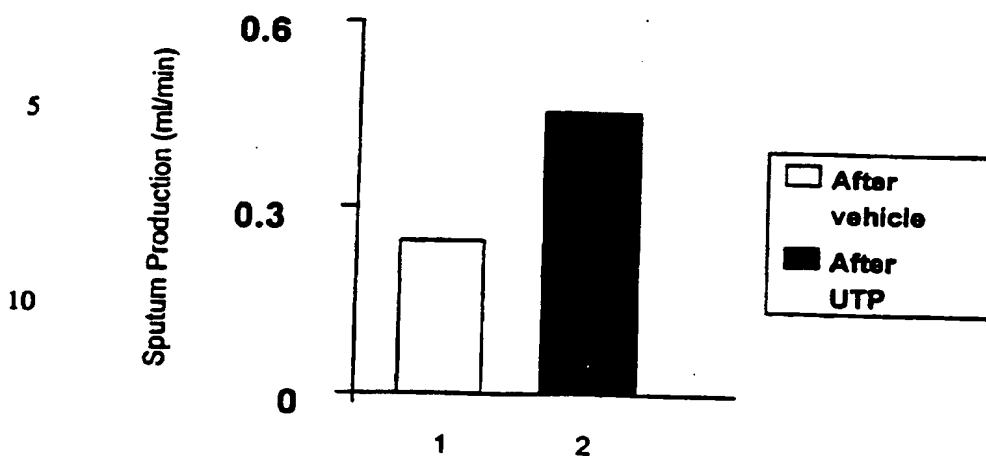


Table II. Volume of sputum produced per unit time in PCD patients

Table II summarizes data demonstrating that the volume of sputum produced per unit time is also enhanced in PCD patients by the inhalation of UTP. The study was a blinded cross-over design and involved 8 patients. Column 1 shows that approximately 0.2 ml of sputum were expectorated per minute following inhalation of saline vehicle. In contrast, Column 2 shows that inhalation of UTP approximately doubled the amount of sputum expectorated in the same time period ( $p < 0.01$ ). Based on data from patient-completed questionnaires (completed in a blinded fashion), UTP appeared to enhance the ease of expectoration relative to vehicle. Several patients stated that sputum was thinner and easier to expectorate following UTP than following vehicle. These data are particularly compelling given that the effect was observed following only a single dose of UTP. With the data in Figure 2, these data indicate that there is a strong correlation between measures of cough clearance and sputum expectoration.

#### Other Embodiments

Other embodiments will be evident to those of skill in the art. although the invention has been shown and described with respect to an illustrative embodiment thereof, it should be appreciated that the foregoing and various other changes, omissions, and additions in the

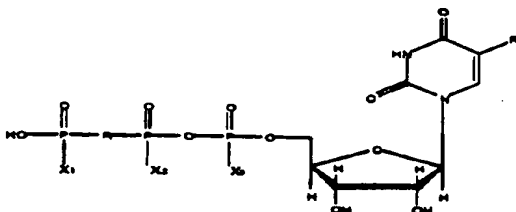
form and detail thereof may be made without departing from the spirit and scope of the invention as delineated in the claims.

WHAT IS CLAIMED IS:

1. A method of stimulating ciliary beat frequency in a subject in need of such treatment, said method comprising:

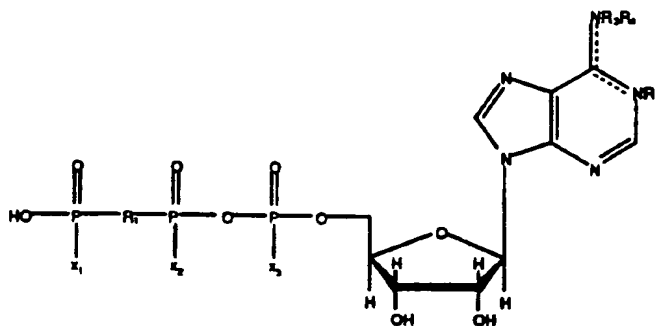
- 5 administering to the subject a compound of Formula I, II, III, or IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to increase ciliary beat frequency in the affected part of the body:

10

Formula I

wherein:

- 15  $X_1$ ,  $X_2$ , and  $X_3$  are each independently selected from the group consisting of OH and SH;  
 $R_1$  is selected from the group consisting of O, imido, methylene, and dihalomethylene; and  
 $R_2$  is selected from the group consisting of H and Br;
- 20

Formula II

wherein:

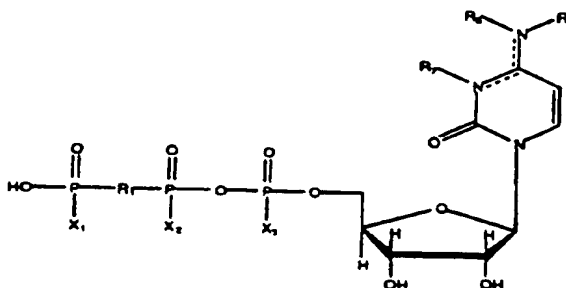
- 25  $R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I,  
 $R_3$  and  $R_4$  are H while  $R_2$  is nothing and there is a double bond between N-1 and C-6 (adenine), or

$R_3$  and  $R_4$  are H while  $R_2$  is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

$R_3$ ,  $R_4$ , and  $R_2$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1, $N^6$ -ethenoadenine);

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### Formula III



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wherein:

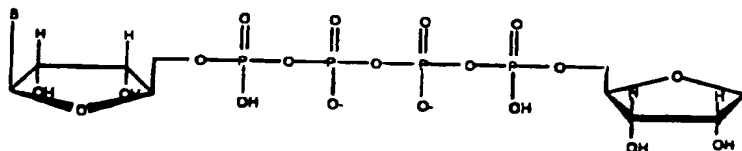
$R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I,

$R_5$  and  $R_6$  are H while  $R_7$  is nothing and there is a double bond between N-3 and C-4 (cytosine), or,

$R_5$ ,  $R_6$  and  $R_7$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3, $N^4$ -ethenocytosine);

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### Formula IV



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wherein:

B is adenine or uracil.

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2. A method according to Claim 1, wherein said compound is delivered to the eyes to treat dysfunction of the ocular mucociliary clearance system as a result of impaired ciliary movement in a patient in need of such treatment.

3. A method according to Claim 1, wherein said compound is delivered to the genito-urinary tract to treat disfunction of the genito-urinary mucociliary clearance system as a result of impaired ciliary movement in a patient in need of such treatment.

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4. A method according to Claim 1, wherein said compound is delivered to the bloodstream to treat immune deficiency caused by impairment of ciliary movement of neutrophils and macrophages in a patient in need of such treatment.

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5. A method according to Claim 1, wherein said compound is delivered to the spermatozoa to treat male infertility caused by impairment of ciliary movement of spermatozoa in a patient in need of such treatment.

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6. A method according to Claim 1, wherein said compound is delivered to the ovaries or fallopian tubes to treat female infertility caused by impairment of ciliary movement of the epithelial cells of the ovaries or fallopian tubes in a patient in need of such treatment.

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7. A method according to Claim 1, wherein said compound is delivered by administering a liquid/liquid suspension, including nasal drops or spray, of said compound to the nasopharyngeal airways of said subject, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries and fallopian tubes, or neutrophils and macrophages of said subject either directly or via systemic absorption and circulation.

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8. A method according to Claim 1, wherein said compound is delivered by administering an oral form of said compound to said subject, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries and fallopian tubes, or neutrophils and macrophages of said subject either directly or via systemic absorption and circulation.

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9. A method according to Claim 1, wherein said compound is delivered by administering an a rosol suspension of said compound to the nasopharyngeal or oral airways of said subject, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries or fallopian tubes, or neutrophils and macrophages of said subject either directly or via systemic absorption and circulation.
10. A method according to Claim 1, wherein said compound is delivered by administering a topical form of said compound to the middle ear or eyes via the tympanic membrane or cornea of said subject, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the luminal epithelial lining of the middle ear or inner ear or the lens of said subject.
11. A method according to Claim 1, wherein said compound is delivered by administering an injected form of said compound, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries or fallopian tubes, or neutrophils and macrophages of said subject either directly or via systemic absorption and circulation.
12. A method according to Claim 1, wherein said compound is delivered by administering a suppository form of said compound, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries or fallopian tubes, or neutrophils and macrophages of said subject either directly or via systemic absorption and circulation.
13. A method according to Claim 1, wherein said compound is delivered by intra-operative instillation of a gel, cream, or liquid suspension form of said compound, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the ears, eyes, lungs, or genito-urinary tract of said subject either directly or via systemic absorption and circulation.

14. A method according to Claim 1, wherein said compound is administered ex vivo to the spermatozoa by means of a topical, injection, or immersion form of the compound, such that a therapeutically effective amount of said compound contacts the spermatozoa having impaired ciliary movement.
15. A method according to Claim 1, wherein said compound is delivered via a transdermal patch, such that a therapeutically effective amount of said compound contacts the area of impaired ciliary movement in the ears, eyes, lungs, or genito-urinary tract of said subject either directly via local absorption or via systemic absorption and circulation.
16. A method according to Claim 1, wherein said compound is administered in an amount sufficient to achieve concentrations thereof on the surfaces of the lungs, sinuses, middle or inner ear, eyes, genito-urinary tract, spermatozoa, ovaries or fallopian tubes, or neutrophils and macrophages or said subject of from about  $10^{-7}$  to about  $10^{-1}$  Moles/liter.
17. A method according to Claim 1, wherein  $X_2$  and  $X_3$  are OH.
18. A method according to Claim 1, wherein  $R_1$  is oxygen.
19. A method according to Claim 1, wherein  $R_2$  is H.
20. A method according to Claim 1, wherein said compound of Formula I is selected from the group consisting of uridine 5'-triphosphate, uridine 5'-O-(3-thiotriphosphate), 5-bromo-uridine 5' triphosphate and the pharmaceutically acceptable salts thereof.
21. A method according to Claim 1, wherein said compound of Formula II is selected from the group consisting of adenosine 5'-triphosphate, 1,N<sup>6</sup>-ethenoadenosine triphosphate, adenosine 1-oxide triphosphate and the pharmaceutically acceptable salts thereof.
22. A method according to Claim 1, wherein said compound of Formula III is selected from the group consisting of cytidine 5'triphosphate



(CTP), 3,N<sup>4</sup>-ethenocytidine triphosphate and the pharmaceutically acceptable salts thereof.

23. A method according to Claim 1, wherein said compound of  
5 Formula IV is selected from the group consisting of P<sup>1</sup>,P<sup>4</sup>-di(adenosine-5')  
tetraphosphate (A<sub>2</sub>P<sub>4</sub>) and P<sup>1</sup>,P<sup>4</sup>-di(uridine-5') tetraphosphate (U<sub>2</sub>P<sub>4</sub>) and  
the pharmaceutically acceptable salts thereof.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US97/05101 <b>(22) International Filing Date:</b> 27 March 1997 (27.03.97) <b>(30) Priority Data:</b> 08/624,914 27 March 1996 (27.03.96) US <b>(71) Applicant (for all designated States except US):</b> INSPIRE PHARMACEUTICALS, INC. [US/US]; Suite 470, 4222 Emperor Boulevard, Durham, NC 27703 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JACOBUS, Karla, M. [US/US]; 200 Riverwalk Circle, Cary, NC 27511 (US). YERXA, Benjamin, R. [US/US]; 1330 Hathaway Road, Raleigh, NC 27608 (US). PENDERGAST, William [US/US]; 5815 Williamsburg Way, Durham, NC 27713 (US). BOUCHER, Richard, C., Jr. [US/US]; 735 Gimghoul Road, Chapel Hill, NC 27514 (US). RIDEOUT, Janet, L., [US/US]; 3101 Morningside Drive, Raleigh, NC 27607 (US). DRUTZ, David, J. [US/US]; 1059 Carterbury Lane, Chapel Hill, NC 27514 (US). JAMES, Michael, K. [US/US]; 4329 Basal Creek Lane, Fuquay-Varina, NC 27526. STUTTS, Monroe, Jackson [US/US]; 104 Norgan Bluff Lane, Chapel Hill, NC 27514 (US). GEARY, Cary [US/US]; 7011 Thurston-Bowles, Campus Box 7248, UNC at Chapel Hill, Chapel Hill, NC 27599 (US).		<b>LAZAROWSKI, Edwardo, R. [US/US];</b> Apartment #3, 212 Pinegate, Chapel Hill, NC 27514 (US). <b>(74) Agents:</b> DREHKOFF, W., Dennis et al.; Banner & Witcoff, Ltd., 10 South Wacker Drive, Chicago, IL 60606 (US). <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i> <b>(88) Date of publication of the international search report:</b> 22 January 1998 (22.01.98)
<b>(54) Title:</b> METHOD OF TREATING CILIARY DYSKINESIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS <b>(57) Abstract</b> <p>A method of stimulating ciliary beat frequency in a subject in need of such treatment is disclosed. The method comprises administering to the airways, ears, eyes, or genito-urinary tract of the subject a triphosphate nucleotide such as uridine 5'-triphosphate (UTP), an analog of UTP, or any other analog, in an amount effective to stimulate ciliary beat frequency. This method is useful for treating patients afflicted with ciliary dyskinesia, Kartagener's syndrome, or any other disease involving dysfunction of ciliary movement, such as male infertility caused by impairment of propulsion of the spermatozoa or immune deficiency caused by impairment of ciliary movement in neutrophils or macrophages. Pharmaceutical formulations and methods of making the same are also disclosed. Methods of administering the same would include any liquid suspension (including nasal spray or nasal or eye drops), oral, inhaled by nebulization, topical, injected, suppository, intra-operative by instillation or application, or ex vivo direct application to spermatozoa.</p>		

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/05101

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 96 40059 A (UNIV NORTH CAROLINA ;STUTTS MONROE J III (US); BOUCHER RICHARD C J) 19 December 1996 * p.4, 1.9-17; claim 16 *	1,7-9, 11-13, 15-23
X	US 5 420 116 A (PUHELLE EDITH ET AL) 30 May 1995 * col.1, 1.16-25; claim 1 *	1,7-9, 11-13, 15-23
X	J. NUUTINEN: "Activation of the impaired nasal mucociliary...." INTERNATIONAL J OF PEDIATRIC OTORHINOLARYNGOLOGY, vol. 10, 1985, pages 47-52, XP000654070 * p.47, Summary and Introduction; p.51, Discussion *	1,7-9, 11-13, 15-23

☒ Further documents are listed in the continuation of box C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>US 5 292 498 A (BOUCHER JR RICHARD C) 8 March 1994</p> <p>* col.1, 1.17-21, 1.50-68; col.3, 1.44-52; claims 1-26, *</p> <p style="text-align: center;">---</p>	<p>1,7-9, 11-13, 15-23</p>
X	<p>DATABASE EMBASE ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL no75036639, XP002035486 see abstract &amp; VOVSJ ET AL: "importance of hydrodynamic indices for pathogenic..." ZDRAVOOKHR.TADZH, vol. 21, no. 3, 1974, pages 35-37,</p> <p style="text-align: center;">---</p>	<p>1,2, 7-13, 15-23</p>
X	<p>DATABASE EMBASE ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL no75207772, XP002035487 see abstract &amp; BUNIN ET AL: "Effects of riboflavin mononucleotide, ATP and drotaverine....." VESTN. OFTAL., vol. 1, 1975, pages 65-8,</p> <p style="text-align: center;">---</p>	<p>1,2, 7-13, 15-23</p>
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Y	<p>DATABASE WPI Section Ch, Week 8741 Derwent Publications Ltd., London, GB; Class B02, AN 87-290829 XP002035488 &amp; SU 1 297 862 A (AS SIBE CYTOLOGY) , 23 March 1987 see abstract</p> <p style="text-align: center;">---</p>	<p>1,2, 7-13, 15-23</p>
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	-/--	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/05101

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO 95 10287 A (WISCONSIN ALUMNI RES FOUND) 20 April 1995 * p.4, 1.34-p.5,1.25; claims 1-8 *	1,4,7-9, 11,12, 16-23
X	M.T. NEWHOUSE: "Primary ciliary dyskinesia: What has it taught us about pulmonary disease?" EUR. J. RESPIR. DIS., vol. 64, no. suppl.127, 1983, pages 151-6, XP002045212 * p.151, 1.4-12 *	1-3,5-9, 11-23
E	WO 97 29756 A (INSPIRE PHARMACEUTICALS INC ;DRUTZ DAVID J (US); RIDEOUT JANET L () 21 August 1997 see the whole document	1,7-13, 15-23
-/--		

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/05101

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>           KNUDSEN ET AL: "Neutrophil function in primary cilia dyskinesia"            EUR J RESPIR DIS,            vol. 64, no. suppl.128, 1983,            pages 476-8, XP002045213            * abstract; p.477, conclusion *            ----         </p>	<p>           1,4,7-9,            11-13,            16-23         </p>
X	<p>           OTTONELLO ET AL: "Neutrophil dysfunction and increased susceptibility to infection"            EUR. J. OF CLIN. INVESTIGATIONS,            vol. 25, 1995,            pages 687-92, XP002045214            * penultimate sentence of the abstract;            Tables 1 and 4 *            -----         </p>	<p>           1,4,7-9,            11-13,            16-23         </p>

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 05101

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 (part.), 2, 7-9 (part), 10, 11-13 (part.), 15-23 (part.)  
1 (part.), 3, 5, 6, 7-9 (part.), 11-13 (part.), 14 and 15-23 (part.)  
1 (part.), 4, 7-9 (part.), 11-13 (part.) and 16-23 (part.)  
(part. = partially)

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/05101

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